

## **Vital Dyes in Vitreomacular Surgery**

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**Abstract:** Vital dyes contain complex molecules with chromophores that stain living tissues and have greatly enhanced identification and removal of transparent vitreoretinal tissues during surgery. Several “chromovitrectomy” dyes are frequently used by vitreoretinal specialists, including indocyanine green, trypan blue, brilliant blue G and triamcinolone acetonide; other dyes are also under investigation. Currently available chromovitrectomy dyes have their limitations, and of particular concern is their possibility for acute and chronic toxicity to the neurosensory retina and retinal pigmented epithelium. The potentially irreversible acute toxicity and other limitations, such as lack of long-term safety profiles, highlight the need for a review of the current literature and for further advancements.

## Introduction

In all surgical fields, visualization of tissues and anatomic planes assumes paramount importance. In vitreoretinal surgery, the surgical planes may be only microns thick, and neighbouring nerve fiber layer tissues are susceptible to mechanical damage with secondary adverse visual sequelae. Thus, surgical dissection in the appropriate plane without traumatizing underlying retinal structures is critical. Particularly challenging in retinal surgery is the visualization of transparent preretinal tissues such as internal limiting membrane (ILM), epiretinal membrane (ERM) and the vitreous cortex. Vital dyes are complex molecules containing chromophores, the structure of a molecule responsible color,<sup>1</sup> that stain living tissues and have greatly enhanced identification and removal of transparent anatomical layers during vitreoretinal surgery.

Currently available chromovitrectomy dyes do have their limitations. The properties of an ideal dye for vitreoretinal surgery include lack of toxicity to all retinal layers, easy application and extraction from the eye, excellent staining and contrast of desired tissues, no potential for phototoxicity, low cost, minimal preparation and existing Food and Drug Administration (FDA) approval. Several chromovitrectomy dyes are frequently used by vitreoretinal specialists, including indocyanine green (ICG), trypan blue (TB), brilliant blue G (BBG) and triamcinolone acetonide (TA); other dyes are also currently under investigation. The 2017 American Society of Retinal Specialists (ASRS) Preferences and Trends (PAT) survey demonstrated that of United States (US) retina specialists survey responders, 69.0% percent preferred to use ICG to aid in ERM and/or ILM peeling, 9.5% preferred TA, 14.8% used BBG, 1.2 % used TB, 3.1% preferred no dye, and 2.4% used an unlisted dye.<sup>2</sup> Additionally, the last decade has seen a trend towards more US providers utilizing ICG, despite some reports of toxicity, with a slight decrease in the use of TA and TB (Figure 1).<sup>2-6</sup>

Of particular concern with chromovitrectomy dyes is the potential for acute and/or chronic toxicity to the neurosensory retina and retinal pigmented epithelium (RPE), and the toxicity of commonly used dyes is discussed throughout this text. While most physicians peel the ILM during macular hole surgery due to the improvement in macular hole closure rates, the visual results of ILM peeling during ERM resection are less convincing. Despite reducing the rate of recurrence of ERMs, ILM peeling has not convincingly improved visual outcomes, and the recurrence rate of ERMs is already relatively low.<sup>7</sup> The potential for harmful effects, varied practice patterns and other limitations highlight the need for a current literature review and further advancements.

### **Historical perspective: Introduction of ICG-assisted ILM peeling**

Surgical repair of idiopathic macular holes has undergone significant evolution in the last three decades. In 1991, Kelly and Wendel published a technique for surgical closure, in which pars plana vitrectomy (PPV) was combined with intraocular gas tamponade and face down positioning. This procedure resulted in 58% macular hole closure rate and 42% improvement in visual acuity (VA), a tremendous improvement compared to observation.<sup>8</sup> Over the ensuing decade, various modifications to this technique were investigated, with the goal of improving closure rates and visual outcomes. The technique that is currently popular involves removal of the ILM around the macular hole. It is hypothesized that ILM removal reduces tangential forces on the fovea, which act as a potential mechanism for failure of macular hole closure. In 1997, Eckardt et al. published a series of 39 full-thickness macular holes that underwent ILM peeling during PPV, and demonstrated a notable improvement in closure rate of 92%.<sup>9</sup> Follow-up studies confirmed these closure rates, effectively establishing the role of ILM peeling in macular hole repair. By 2014, the ASRS PAT survey noted 93% of

responding US retinal physicians performed routine ILM peeling during macular hole surgery.<sup>5</sup>

One of the technical difficulties in peeling the ILM is adequate visualization of this thin transparent membrane, with concern for damage to the inner retinal nerve fiber layer and permanent scotoma from excessive manipulation. Challenges in consistent visualization of ILM led investigators to evaluate ICG as a dye to stain this transparent layer. ICG is a water-soluble, tricarbo-cyanine dye with infrared absorption properties that was initially used as a contrast agent in radiology. Prior to its use for ILM peeling, ICG has been used for imaging of the choroidal circulation and was also investigated for staining of the anterior lens capsule in cataract surgery.<sup>10</sup> A favorable safety profile for ICG was suggested by the lack of adverse effects when it was used for dye-based angiography, even in cases where large amounts of ICG dye leaked into or under the retina.<sup>11,12</sup> For surgical use, ICG in the form of a powder must be dissolved in sterile water and diluted to the desired concentration.

In 2000-2001, teams in both Japan and the US evaluated ICG-assisted ILM peeling in animal models, and the preliminary safety and success of these preclinical studies led to human trials. In humans, following PPV and PVD induction, the ICG was applied directly to the ILM for several minutes and was then aspirated. The staining provided enhanced visualization of the ILM and aided in its peeling. The resulting supportive publications with excellent images, along with the conceptual appeal, good outcomes and early lack of adverse events generated positive response within the vitreoretinal community.<sup>13,14</sup>

Over the ensuing years, various groups replicated the successful staining demonstrated in these original studies.<sup>15-17</sup> However, case reports of potential toxicity related to ICG soon surfaced, including reports of worse visual acuity outcomes and

peripheral visual field defects.<sup>18-22</sup> Retinal pigmentary changes were observed in up to 50% in some series and reports of RPE atrophy surfaced as well.<sup>23-25</sup> However, the development of RPE changes following PPV may be unrelated to the use of ICG, as the Vitrectomy for Macular Hole Study Group in 1997 was performed prior to the application of ICG or other dyes, and noted that 33% of patients developed RPE changes.<sup>26</sup> In 2001, Gandorfer et al. worked on elucidating the potential reasons for these adverse outcomes by performing electron microscopy on excised ILMs. To their surprise, microscopy revealed that, in addition to ILM removal, plasma membranes of Muller cells, Muller cell foot plates and other undetermined cellular debris were excised.<sup>18</sup> In contrast, ILM peeling without the assistance of ICG, did not reveal excessive excision of non-ILM tissue.<sup>27</sup>

While the exact mechanisms for ICG-related injury to retinal tissue remains controversial, studies suggest that ICG has direct toxicity to the RPE, especially if utilized at higher concentrations and over longer periods of contact during surgery. The hypothetical mechanisms for ICG-related RPE and neurosensory retinal damage are summarized in Table 1, along with suggested techniques to minimize damage.

Conflicting rates of these adverse outcomes have been published in larger studies,<sup>22,28-31</sup> variations in ICG concentration, addition of dextrose 5% in water (D5W) to the diluent, ICG exposure time, endoillumination time, surgeon experience and the degree of irrigation confound comparison of different studies. Possibly the best evidence to date is a 2012 meta-analysis of both retrospective and randomized, prospective studies. This analysis compiled the results from twenty-two studies and 1585 eyes, and concluded that ICG-assisted ILM peeling was associated with increased rates of visual field defects and worse visual acuity outcomes compared to ILM peeling without ICG. In particular, the percentage of patients who improved to better than

20/40 was lower in those with the ICG-assisted peeling compared to the those without ICG (OR 0.61, 95% CI 0.43 to 0.97,  $p=0.033$ ), despite similar macular hole closure rates.<sup>32</sup> Based on the potential adverse effects of ICG, there continues a search for alternative dyes for routine staining of the ILM. Alternatively, steps should be taken to minimize the concentration, exposure time and illumination during surgery.

**Table 1. Hypothetical mechanisms for indocyanine green (ICG)-related tissue damage and suggested techniques to minimize this damage.**

Hypothetical mechanisms for ICG-related tissue damage	Suggested techniques to minimize damage
Dose- and time-dependent direct toxicity to RPE <sup>33,34</sup>	Minimize the dose (0.5mg/mL) and minimize exposure time during surgery. <sup>35</sup> Copiously irrigate ICG to minimize chronic direct toxicity. Avoid direct injection to the macular hole or use various materials (ex: viscoelastic material) to create a barrier between ICG and RPE. <sup>36,37</sup>
Acute and chronic phototoxicity <sup>38</sup>	Minimize direct and proximal light-pipe illumination during surgery. <sup>39</sup> Copiously irrigate ICG to minimize chronic phototoxicity. Dissolving the dye in BSS or D5W alters the absorption spectrum and can minimize phototoxicity. <sup>40</sup>
More aggressive surgical excision <sup>41,42</sup>	Minimize manipulation of ILM and underlying neurosensory retina. However, the aggressive surgical excision may in part be due to ICG chemically modifying the surgical plane.
Hypo-osmotic effects (controversial) <sup>33,43,44</sup>	Utilize a physiologic osmolarity

BSS, balanced salt solution; D5W, 5% dextrose in water; ICG, indocyanine green; RPE, retinal pigment epithelium

This table describes the hypothetical mechanisms for ICG-related retina toxicity and techniques to minimize potential toxicity.

ICG has separately been investigated to facilitate epiretinal membranesurgery. While several studies have confirmed poor ERM staining with ICG as highlighted in Figure 2, the negative ICG staining of ERM accompanied by staining of adjacent ILM may facilitate removal of both ERM and ILM combined.<sup>45,46</sup> Negative staining with ICG and other dyes, wherein the lack of staining of the tissue of interest, contrasted by staining of adjacent tissue, improves visualization and can aid in excision. Negative staining techniques have been applied to the removal of vitreous, posterior hyaloid, ERM and ILM.<sup>47</sup>

Other dyes such as TB and triamcinolone, discussed later in this review, more favourably stain ERMs. Finally, infracyanine green (IFCG), a biosimilar dye to ICG, was briefly investigated as an alternative to ICG and was hypothesized to have an improved safety profile due to a more physiological osmolarity as well as a lack of iodine.<sup>48-52</sup> After several published investigations, alternate dyes such as trypan blue and brilliant blue G gained more investigative enthusiasm and IFCG is not routinely used.

### **Brilliant Blue G**

Brilliant blue G, also known as acid blue 90 and Coomassie BBG, is a blue dye that non-selectively binds to most proteins and has been utilized for protein staining in biologic fields. The potential toxicity of ICG led investigators in Japan to evaluate the



safety of this dye. In 2006, Enaida et al. published a thorough evaluation of various doses of BBG in vitrectomized rat eyes. Following successful preclinical evaluation, BBG was investigated in humans as a dye for ERM and ILM peeling (Figure 3). In 2006, Enaida et al. published the successful application of 0.25 mg/ml of BBG during epiretinal membrane resection and ILM peeling for macular hole repair in a small group of eyes. No BBG-related adverse events were noted, but peripheral visual field evaluation was not performed. Similarly, ERMs were well-visualized and effectively peeled, without documented BBG-related adverse events. Follow-up studies confirmed efficacy of BBG in ILM staining, with minimal observed toxicity.<sup>53</sup> A recent review and meta-analysis of 846 eyes demonstrated the superior visual outcomes of BBG compared to ICG for ILM peeling in macular hole surgery.<sup>54</sup> Long-term follow-up, of an average of 2 years following surgery, similarly demonstrated superior visual acuity outcomes for BBG over ICG.<sup>55</sup>

Limitations of BBG include the possible subjective and objective inferiority of ILM staining compared to ICG, lack of FDA approval, minimal staining of ERMs and reports of adverse events.<sup>56</sup> Despite its possible inferior staining, as compared to ICG, the dye's widespread use suggest adequate ability to aid in visualization of the ILM. The number of adverse events reported with BBG is fewer than with ICG.<sup>57</sup> On examination of excised ILMs following the application of various dyes, BBG had similar findings as TB, BPB and CB, and specimens did not contain large cellular fragments and Muller cell end-feet as was found with ICG derived ILM specimens.<sup>58</sup> The clinical significance of the excision of a small amount of retinal tissue still remains to be elucidated. Similar to the use of ICG, greater light intensity and duration, as well as higher concentrations of BBG and contact time, have been demonstrated in vivo to

result in cell toxicity; thus, application dose and duration should be minimized during surgery.<sup>59</sup>

Currently, approximately 14.8% of US providers utilize BBG for aid in ILM peeling.<sup>2</sup> For surgical use, it can be purchased as a solution that is then diluted to a desired concentration. It is commercially available as Brilliant Peel® (Fluoron, Ulm, Germany) and Ocublue Plus® (Aurolab, Madurai, India). It is available in combination with trypan blue as Bio-Blue DUO® (Biotech Visioncare, Gujarat, India) and Membrane Blue-Dual® (Dutch Ophthalmic Research Center, Zuidland, Netherlands), and with trypan blue and lutein as Doubledyne®, Tripledyne® and Retidyne® (Kemin Industries, Inc., U.S.A). Overall, BBG appears to have fewer safety concerns than ICG but, it has slightly inferior staining properties, is not FDA approved, requires compounding and the long-term adverse and visual field effects have not been thoroughly evaluated.

### **Triamcinolone Acetonide**

Triamcinolone acetonide (TA) is a sterile, corticosteroid, suspension that is used in one of two forms for vitreoretinal surgery: Non-preservative free triamcinolone acetonide (non-PFTA), commercially available as Kenalog® (Bristol-Myers Squibb, NJ) or preservative free triamcinolone acetonide (PFTA), commercially available as Triescence® (Alcon, Fort Worth, TX). Non-PFTA is formulated in a vehicle that contains 0.99% benzyl alcohol as a preservative. While its intraocular use is off-label, non-PFTA was the initial formulation used for intravitreal injection for macular edema and then used as an adjuvant during PPV.<sup>60</sup> There are concerns about the preservative in non-PFTA which has produced in vitro retinal cell toxicity. Thus, PFTA was developed as an alternative, and is an FDA approved corticosteroid for intraocular use. It is

available as a 1 mL vial with a concentration of 40 mg/mL. Advantages of PFTA include its FDA-approved status for intraocular use (Triescence) and the lack of preservatives, while non-PFTA is more cost-effective. Currently, approximately 9.5% of US providers utilize TA for aid in peeling the ERM or ILM.<sup>2</sup>

Within vitreoretinal surgery, TA can be utilized during PPV, where it adheres to vitreous to enhance visualization. It facilitates visualization of vitreous cortex and the separation of the posterior hyaloid, tissues that can be challenging to visualize due to their transparency. Upon administration into the vitreous cavity, the compound becomes trapped within the vitreous gel, where the white steroidal crystals provide contrast with adjacent tissues. Since 2000, the use of TA for vitreous visualization rapidly gained acceptance due to this effective coating ability..<sup>61,62</sup>

In addition to the effective staining of vitreous, TA has also been investigated for other surgical applications. The corticosteroid coats the ILM, possibly due to residual collagen fibers on the transparent tissue following hyaloid separation.<sup>63,64</sup> As opposed to staining with dyes such as ICG, TA coats tissues allowing for visualization of peeled membranes.<sup>65</sup> Retrospective analyses of TA application in MH repair has not shown toxicity to the retina or RPE, in contrast to the early ICG for macular hole surgery studies.<sup>55,66</sup> Furthermore, in select studies, the visual outcomes with the use of TA were superior to the use of ICG.<sup>67</sup> Additional applications include the coating of ILM and ERM in PVR resection.<sup>68</sup>

With regard to safety, TA use during vitrectomy has been inconsistently associated with postoperative ocular hypertension.<sup>66,69</sup> The conflicting results are likely due to the independent ocular hypertensive effects of PPV as well as the use of gas and periocular or topical corticosteroids.<sup>66,67</sup> The rates of ocular hypertension are significantly lower than following intravitreal depot injection because most TA is

washed out during PPV. However despite irrigation, several studies have found residual TA on or beneath the retina. Fortunately, no local untoward effects of this residual TA have been demonstrated, likely due to its indolent nature and relatively short half-life in ocular tissue (18.6 days in nonvitrectomized eyes and 3.2 days in vitrectomized eyes), with the residual TA following vitrectomy typically absorbed by 8 weeks.<sup>67,70-73</sup> Studies have not shown increased rates of endophthalmitis or cataract formation with the use of TA during PPV despite the cataractogenic and immunosuppressive nature of corticosteroids.<sup>66</sup>

Overall, TA is a useful adjuvant to coat the vitreous cortex during PPV and facilitate posterior hyaloid separation. Additionally, its coating ability facilitates ERM and preretinal membrane peeling. It does not stain ILM to the extent of the other vital dyes which truly stain these tissues, and thus does not enhance visualization to the same degree.

### **Trypan blue**

Trypan blue (TB) is an anionic, hydrophilic azo dye. After favourable safety data following its use in the anterior segment as an anterior capsular stain, it has been evaluated in vitreoretinal surgery to stain epiretinal membranes and the ILM.

Favourable preclinical safety studies led to the first investigation of TB as an aid to ERM peeling in proliferative vitreoretinopathy.<sup>74</sup> Shortly thereafter, TB dye demonstrated efficacy staining the ILM during macular hole surgery.<sup>75</sup> Numerous studies indicate that TB stains both the ERM and ILM,<sup>75-80</sup> but other authors contend that TB does not effectively stain the ILM.<sup>81,82</sup>

Some safety concerns have arisen although not to a similar magnitude as with ICG. Similarly to ICG studies and the natural history following macular hole surgery

without the use of vital dyes, several publications have noted development of RPE atrophy following macular hole surgery using adjuvant TB.<sup>83,84</sup> Histopathologic studies evaluating TB-surgically removed ILM specimens show variable amounts and sizes of glial elements, although typically less fragments than with ICG.<sup>58,85,86</sup> This variability in results may be related to differences in aggressiveness of surgical excision and lack of standardization in evaluation of surgical specimens. Regardless, the clinical relevance of these neural elements on excision remains controversial, as the effect of excising non-ILM tissue during membrane peel has not been consistently demonstrated to have functional sequelae. For example, Li et al. found that despite removal of the neural elements with membranes, patients still had appropriate visual outcomes.<sup>85</sup>

A prospective, randomized trial by Haritoglou suggested an acceptable safety profile for TB without the visual field defects, RPE changes or suboptimal visual acuity outcomes that were observed after ICG-assisted ILM peeling.<sup>87</sup> In a direct prospective, non-randomized comparison of IFCG and TB-assisted peeling of the ILM, TB had better central visual field results.<sup>51</sup> In contrast, a prospective, randomized study, investigating a larger number of patients, found no difference in visual field or visual acuity outcomes between ICG- and TB-assisted ILM peeling in macular holes.<sup>88</sup> These two studies provide the best quality evidence comparing TB with other dyes. Overall, they are suggestive of good central visual acuity and peripheral visual field outcomes with the use of TB, with possible but not definitive superiority over ICG.

Trypan blue (TB) was approved by the FDA in 2009 for epiretinal membrane removal and is available as MembraneBlue 0.15%® (Dutch Ophthalmic USA). Of note is that the TB concentration approved for vitreoretinal surgery is 0.15% and differs from that of VisionBlue 0.06% which is approved for staining of the anterior lens capsule. Despite FDA approval, only 1% of US providers utilize TB for ERM and/or ILM

removal as per the 2017 ASRS PAT survey.<sup>2</sup> Despite possibly fewer adverse events compared to ICG as well as FDA approval, the lack of popularity of TB stems from inferior ILM staining compared to the alternative dyes which narrows its applications.

### **Investigational Dyes**

Numerous dyes have been proposed as alternatives to those currently used, with the ultimate goals of minimizing toxicity and maximizing visualization at minimal cost. Since 2004, most investigational dyes haven't advanced past preclinical trials and a few human case series, including Congo Red,<sup>89</sup> Chicago Blue,<sup>90-92</sup> E68,<sup>91,93</sup> Evans Blue,<sup>89,94-96</sup> Fast Green,<sup>89,94-97</sup> Fluorometholone acetate,<sup>98</sup> Indigo Carmine,<sup>96</sup> Light Green,<sup>89</sup> Methyl Violet,<sup>89,99,100</sup> Methylene Blue,<sup>89,94</sup> Orangell,<sup>99</sup> Patent Blue V,<sup>92,101-106</sup> Rhodulinblua-basic 3,<sup>90,99</sup> Rhodamine 6G,<sup>90,107</sup> Sudan Black,<sup>89</sup> Toluidine Blue<sup>89</sup> and Trisodium.<sup>99</sup>

Other dyes have been investigated to a greater degree, either historically or currently. Bromophenol Blue underwent extensive preclinical evaluation<sup>95,97,108</sup> and the blue dye results in moderate staining of ERM, ILM and vitreous.<sup>89,109</sup> Despite in vitro suggestion of delayed toxicity,<sup>110</sup> the dye has been investigated in humans without evident toxicity,<sup>58,111</sup> and is commercially available at a concentration of 1.3 mg/ml with BBG as Brilliant Peel Dual Dye® (Fluoron, Ulm, Germany).

Infracyanine green, was a promising alternative to ICG due to a physiologic osmolarity, hypothetically less cytotoxicity due to a lack of iodine, and less phototoxicity due to a higher peak wavelength absorption spectrum.<sup>40</sup> In vitro studies supported this hypothesis and demonstrated less cytotoxicity to cultured RPE and retinal ganglion cells in comparison to BBG, ICG And bromophenol blue.<sup>108</sup> Animal studies<sup>112</sup> and subsequently human trials suggested good visualization of ILM without obvious

toxicity.<sup>48,50,113</sup> Further investigation of this dye has been surprisingly limited, with the last publication in 2013.<sup>95</sup> The reasons for the possible decline of investigative fervour include modifications to minimize the toxicity of ICG, the regulatory approval of other dyes and experience suggestive of better outcomes with the use of other dyes (personal communication with investigator, unpublished data).

Lutein is a yellow-orange dye that is analogous to TA in that it coats intraocular tissues, in contrast to true staining. Lutein highlights the vitreous well but only coats ILM and ERM mildly. However, when combined with other dyes it hypothetically reduces cytotoxicity. The dye appears safe in preclinical<sup>114,115</sup> and human studies<sup>116</sup> and is commercially available for vitreoretinal use as Retidyne®, Retidyne Plus® and Vitreodyne® (Kemin Industries, Inc., U.S.A).

Acai fruit is the most promising of a group of naturally occurring dyes that have recently been investigated.<sup>117,118</sup> It is a purple-colored anthocyanin dye that preferentially stains the ILM and recently underwent human trial (Clinicaltrials.gov: NCT02691429) with the results yet to be published.

The introduction of Acid Violet to the market provides a cautionary tale. Preclinical evaluation suggested lack of toxicity at concentrations up to 0.125 mg/ml,<sup>119,120</sup> and some concern for phototoxicity.<sup>92</sup> Ala Medics introduced Ala Purple to the market at a concentration of 1.5 mg/ml. Shortly thereafter, several publications suggesting the dye not be used due to the toxicity at commercially available concentrations,<sup>121-123</sup> and the company pulled the product from the market. Subsequent in vitro studies further supported the toxicity profile of Acid Violet.<sup>124</sup>

If dyes pass preclinical evaluation, careful evaluation in humans is required with focus on visual fields, retinal nerve fiber layer evaluation in addition to the traditional structural OCT analysis of the vitreomacular interface. For broad acceptance and

application, novel dyes have a major hurdle to demonstrate an interval improvement in staining and safety over those currently available. However, if less expensive to produce and distribute, they may only need to demonstrate similar safety and efficacy to become established in the market. A paucity of studies exists directly comparing commonly used dyes or comparing to ELM removal or macular hole surgery without the use of dye. Factors to evaluate include long term visual field and OCT retinal nerve fiber layer outcomes, and standard outcomes including macular hole closure rate, epiretinal membrane recurrence, visual acuity, macular OCT changes and development of RPE atrophy.

## **Conclusion**

Vital dyes are used to facilitate excision of transparent intraocular tissues. A comparison of commonly used dyes is presented in Table 2. TA is an excellent option to stain residual vitreous and the posterior hyaloid. TA can delineate ERM and facilitate removal. However, it is inferior to stain ILM compared to other options. ICG is broadly used in vitreoretinal surgery, but concerns exist about ICG toxicity to the RPE and neurosensory retina with potential visual sequelae. It is unclear whether techniques to minimize ICG exposure, including limiting duration of contact, minimizing concentration, utilizing D5W as a solvent, avoidance of application to the macular hole and thorough irrigation following application are able to completely eliminate potential for toxicity . New dyes such as BBG and novel dyes under development, may ultimately prove superior with regards to efficacy and safety.



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## Figure Legends

**Figure 1. US retina specialist ILM/ERM removal dye preference over time.** The data, obtained from annual ASRS preference and trends surveys, demonstrates an increasing popularity of ICG for ILM and/or ERM peeling over the last decade, and a trends towards a decrease in the use of TA and TB. BBG, brilliant blue; ERM, epiretinal membrane; ICG, indocyanine green; ILM, internal limiting membrane; TA, triamcinolone acetonide; TB, trypan blue. The data for this graph was obtained and used in this manuscript with permission from the ASRS. ASRS, American Society of Retina Specialists; BBG, Brilliant Blue G; ERM, epiretinal membrane; ICG, indocyanine green; ILM, internal limiting membrane; TB, trypan blue; US, United States.

**Figure 2: Indocyanine green staining of epiretinal membrane (ERM) and internal limiting membrane (ILM).** In image plane A, the poor staining of ERM is demonstrated by the translucent ERM tissue visualized over the contrasting macular

hole (white arrow). In contrast, in image B, the excellent staining of ILM is readily visualized (black arrow). Photographs courtesy of Dr. Caroline Baumas.

**Figure 3. Brilliant Blue G (BBG) staining of the internal limiting membrane (ILM) in idiopathic macular hole repair.** The blue staining of the ILM with BBG provides excellent contrast with the underlying neurosensory retina and surrounding ILM.

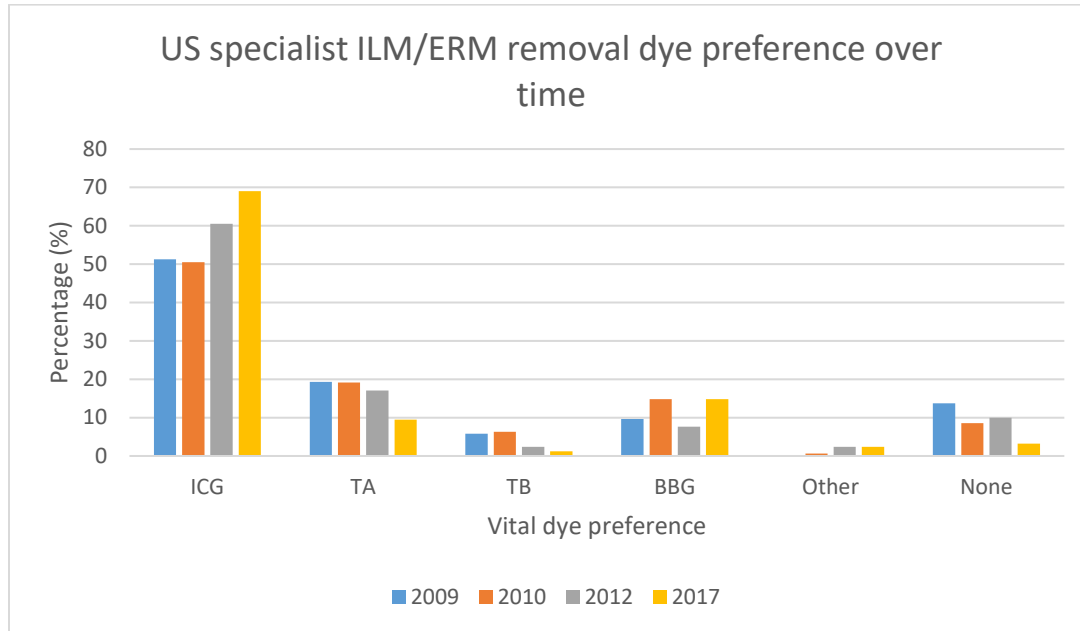
Photograph courtesy of Dr. Thomas Ciulla.

**Table 3. Comparison of various dyes, their ability to stain transparent ocular tissues and safety concerns.**

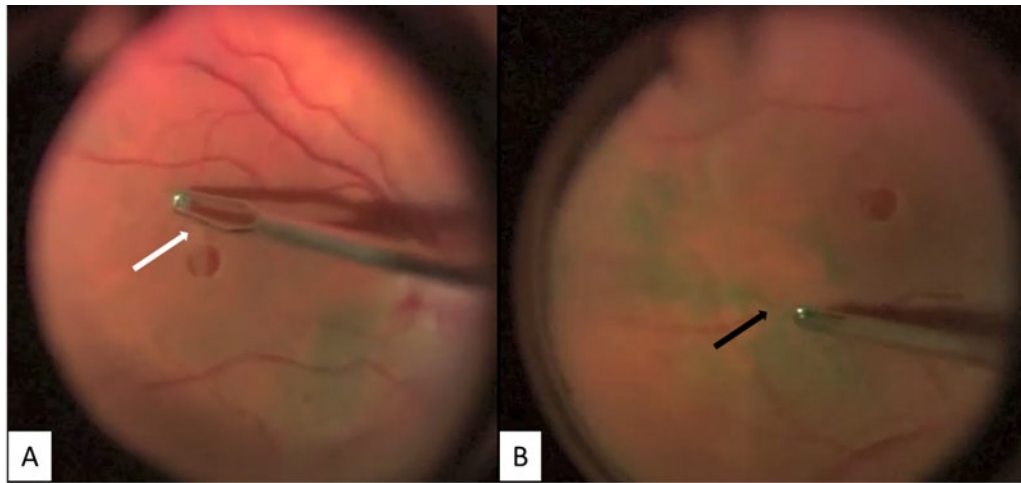
<b>Dye</b>	<b>Staining of ILM</b>	<b>Staining of ERM</b>	<b>Staining of vitreous</b>	<b>Safety concerns</b>
Indocyanine green	+++	+	+	+++
Trypan Blue	+	+++	+	+
Triamcinolone Acetonide	+	+	+++	+
Brilliant Blue G	++	+	+	+

This table provides a comparison of various dyes in their ability to stain transparent ocular tissues and safety concerns. ILM, internal limiting membrane; ERM epiretinal membrane; +, minimal; ++, moderate; +++, maximal.

**Figure 1. US retina specialist ILM/ERM removal dye preference over time.**



**Figure 2: Indocyanine green staining of epiretinal membrane (ERM) and internal limiting membrane (ILM).**



**Figure 3. Brilliant Blue G (BBG) staining of the internal limiting membrane (ILM) in idiopathic macular hole repair.**

